Mosquito Surveillance Activities Plan

I. Introduction

Mosquito surveillance, along with bird-based surveillance, should be a mainstay of regional surveillance programs for West Nile virus (WNV). An effective mosquito surveillance program provides an estimate of vector species abundance and distribution. This data is used to estimate risk levels, guide control operations and to evaluate control methods. Laboratory testing of mosquitoes for arbovirus infection provides information on the relative risk to humans and animals. Mosquito surveillance is best used in conjunction with avian surveillance because birds are often the first sign of WNV activity in any area. Concentrations of infected birds serve as an indication of where to target intensive mosquito surveillance activities.

A universally applicable arbovirus surveillance system does not exist, thus, local mosquito surveillance systems should be tailored according to (1) the probability of arbovirus activity, and (2) the resources available for surveillance. In Virginia, mosquito control is administered locally, either by mosquito control commissions, public works departments, or by district health departments. Therefore, the capabilities and nature of vector control varies from locality to locality, and as mosquito surveillance is an integral part of control, it will also vary with program capabilities. Urbanized areas of the southeastern region of Virginia have had established mosquito control programs for many years, and these programs have the most developed and experienced surveillance programs in the state. Most surveillance programs in the southeastern region of the state have adopted arboviral surveillance methodologies. The methodologies used by these districts were designed initially to detect Eastern Equine Encephalitis (EEE) virus, but more recently have been adapted to WNV surveillance. This plan addresses practices that can be used by existing mosquito surveillance programs to expand and adapt their surveillance capabilities for WNV surveillance. It will also serve as a guide for the establishment of vector mosquito surveillance programs in regions of the state where little or no vector control work has been done.

II. Objectives

This protocol for mosquito surveillance, will serve as guidance for local jurisdictions that are planning to conduct mosquito and arboviral surveillance. The goals of mosquito surveillance are numerous and serve to obtain the following types of information about their local mosquito populations:

1) Identifying the mosquito species that are present in a region;
2) Identifying the mosquito species that are the cause of local citizen complaints, and determining whether they are important WNV vector species;
3) Identifying and mapping mosquito breeding habitats for larval control purposes;
4) Defining the geographic area affected by mosquitoes originating from identified habitats and the geographic area that needs to be treated to control adult mosquitoes;
5) Estimating the population density of a local mosquito species and the desired trigger threshold (population density) for initiating control;

6) Determining when local mosquito populations are at an appropriate developmental and/or behavioral stage to apply control measures;
7) Determining the effectiveness of local mosquito control measures;
8) Determining whether vector mosquito species are present in an area, and whether they are infected by WNV and/or other arboviruses;
9) Determining the mosquito infection rate (MIR) for WNV or other arboviruses in a vector species population; and
10) Determining the seasonal activity patterns of local mosquito species;

III. Implementation Plan

A. General

The VDH Public Health Entomologist will coordinate and encourage collaboration among a network of mosquito control professionals, public health entomologists and vector control organizations in Virginia to carry out mosquito surveillance and to assist in the education of government officials and personnel involved in vector surveillance and control. The Entomology Department at Virginia Tech will provide technical advice and assist in surveillance activities in southwestern and western portions of Virginia. The Virginia Mosquito Control Association (VMCA) will offer technical, educational and organizational services to mosquito surveillance and control professionals throughout the state. VDH and the Virginia Interagency Arbovirus Task Force will develop and disseminate yearly updates of the WNV Response Plan. Regions of the state and their associated mosquito surveillance programs are listed below.

1. **Eastern Region** – Mosquito surveillance operations in this region are conducted by the following Health Districts and organizations: Norfolk Department of Public Health, Division of Vector Control; Virginia Beach Mosquito Control; Chesapeake Mosquito Control; Suffolk Mosquito Control; Portsmouth Mosquito Control; Hampton Mosquito Control; Newport News Mosquito Control; York County Mosquito Control; James City County Mosquito Control; Langley Air Force Base Mosquito Control; Gloucester County Mosquito control; Mathews County Mosquito Control and the Eastern Shore Health District. Personnel from many of the above organizations belong to the Tidewater Regional Arbovirus Surveillance Team (TRAST), and/or the Virginia Mosquito Control Association (VMCA), which play key roles in coordinating and supporting vector surveillance and control activities in the southeastern region.

2. **Southwest Region** – Mosquito surveillance in this region is being conducted by the following Health Districts and organizations: New River Health District; Lenowisco Health District; Allegheny Health District; and Virginia Tech Department of Entomology (Medical Entomology Section).

3. **Northern Region** – Mosquito surveillance in this region is being

conducted by the following Health Districts and organizations: Alexandria City Health Department; Arlington County Health Dept.; Fairfax County Health Department; Loudoun County Health Department; and Prince William County Mosquito Control.

4. **Northwest Region** – Mosquito surveillance in this region is being conducted by personnel from the Lord Fairfax Health District.

5. **Central Region** – Mosquito surveillance in this region is being conducted by the following Health Districts and organizations: Richmond City Health Department, Chesterfield Cooperative Extension Service; and the Henrico County Extension Service.

In addition to the above listed organizations involved in mosquito surveillance, the VDH - Office of Epidemiology hires two seasonal mosquito biologists each summer to conduct mosquito surveillance around the state. These biologists are under the supervision of David Gaines, Ph.D. (VDH-Public Health Entomologist), and operate out of a laboratory space within the State Division of Consolidated Laboratory Services (DCLS) laboratory in Richmond. They are available to conduct investigative surveys of mosquito populations and arboviral events in any jurisdiction that requests their assistance. Although they have the capability to investigate WNV events in any part of the state, they are only available for special investigations, and cannot serve as a substitute for a season-long, local mosquito surveillance program. VDH strongly recommends that jurisdictions needing mosquito surveillance capabilities work towards the development their own surveillance personnel and capabilities.

B. Surveillance Methods

Mosquito Surveillance involves numerous different strategies and practices. A variety of different methods are used to trap mosquitoes in the field because different mosquito species have different behavior and biology and cannot all be collected by the same method. For example, some mosquito species are readily caught in traps whereas other species are rarely collected in traps. Different types of traps are used for different species of mosquitoes. Also, larval mosquitoes occupy a different environment than adult mosquitoes, so collection methods used for larvae are much different than those used for adults. For WNV, EEE or LAC surveillance, appropriate species of adult mosquitoes should be collected, pooled and submitted to the laboratory for arboviral testing. Surveillance should be utilized for determination of arboviral risk as well as for planning, execution, and evaluation of control practices.

1. **Larval Surveillance -** Surveys of immature mosquitoes are an important aspect of any surveillance program, and for certain species, larval surveillance may be a more accurate measure of mosquito population density than adult trapping. Larval surveillance is essential for the

appropriate targeting of larval control methods. Larval surveillance should begin early in the season, even before adults are active to help identify the breeding sites of vector species so that larval control efforts can be targeted. Larval surveillance can be conducted as part of inspection and complaint investigation activities and is often done in conjunction with the application of larvicides for control. In areas where there is no baseline mosquito surveillance data, larval samples can be used to identify and map vector-breeding sites. This information can then be used to help in determining appropriate trap locations to monitor adult mosquito populations.

a. Yard Inspections: Whenever an environmental health person or mosquito control staff member is in a private yard around a home, that person should observe the area, looking for all potential mosquito breeding habitats (i.e. barrels, buckets, tarps, boats, ornamental ponds, old appliances, toys, trash, or any other item that may hold as little as a tablespoon of water). When infested habitats are encountered they can be dipped for larval samples. Samples can be poured into whirl-pack or zip-lock bags and returned to the office for larval identification or maturation to adult stage for identification. Larval habitats can be dumped, but if homeowners are home, habitats and larval samples should first be shown to the homeowner for their own edification. This provides an opportunity for educating the property owner or occupant on what mosquito larvae look like and how to eliminate a potential or existing mosquito problem.

b. Equipment, Techniques and Record Keeping: Larval surveillance requires the use of minimal and inexpensive equipment. Equipment should include: a long handled dipper; a small soup ladle (for dipping into tires or small holes); a small white, plastic or enamel pan (to dump dip samples into for close observation and detection of very small larvae); a turkey baster (for sample transfer; Whirl-pak ® larval collection bags (for collection of larval samples); a tea strainer (used to pour off excess water to concentrate larval samples); and a shoulder bag (to carry equipment in). Larval surveillance may require the use of different dipping techniques depending on the target species and habitat (see Attachment 3.A). Accurate records should be kept of when and where larvae are collected (see Larval Surveillance Data Form; Attachment 3.A).

2. **Adult Surveillance** - Because it is the adult female mosquito that carries and transmits diseases, many surveillance techniques have been devised to collect adult female mosquitoes to monitor or record their activities. Techniques include the use of trapping, mechanical aspirator collections,

and documentation of mosquito activity through citizen complaints. Trapping is widely used, but day-to-day success may be variable due to variation in environmental conditions such as wind, air temperature, rainfall and trap location. Several different types of traps are used and each type is used to trap certain species of mosquitoes. There are also certain mosquito species that will not be attracted to traps and which must be collected by some alternative means. It is often advisable to use several types of traps (e.g., gravid traps and CDC-light traps) at a single trap site to collect a representative sample of the species active at that location. Data on the trapped mosquitoes should be maintained to create a historical record of mosquito species found in association with different habitats in different parts of a jurisdiction. Trapped mosquitoes that have been identified can either be logged into the computerized mosquito database (see section B.5 below), or may be logged onto a paper data sheet for future data entry (see Weekly Adult Mosquito Trap/Collection Form, Attachment 3.B). The most common trapping and adult collection and monitoring methods used include:

a. Reiter gravid trap – The Reiter gravid trap is designed to collect gravid mosquitoes and is among the most important mosquito traps used for WNV surveillance. Gravid mosquitoes are mosquitoes that are carrying eggs and are seeking a place to lay them. The gravid trap was originally developed for monitoring mosquitoes in the *Culex pipiens* complex for St Louis Encephalitis surveillance. Gravid traps will also work for trapping several of the *Aedes* and *Ochlerotatus* species that breed in containers and may be important arbovirus vectors (e.g., WNV, EEE, LAC). Gravid traps are the most effective means of collecting *Culex pipiens* and *Cx. restuans* which are the most important "primary vectors" of WNV (primary vectors are those species responsible for transmitting WNV to the bird population). The container breeding *Ochlerotatus* and *Aedes* species captured in gravid traps include: the Asian tiger mosquito (*Aedes albopictus*), the Eastern tree-hole mosquito (*Ochlerotatus triseriatus*), and the newly introduced Asian rock-pool mosquito (*Oc. japonicus*). These species are potentially among the most important "bridge vectors" for WNV and LAC (bridge vectors are those species which can bite birds, and commonly bite humans or other mammals and serve as a bridge for the virus to move from bird to mammal). *Ae. albopictus* might also be an important EEE vector.

   Gravid traps use a small electric fan, typically powered by a 6-volt lantern battery to suck up the mosquitoes that visit the bait container, and blow them into a collection bag. Gravid traps are baited with a tub of smelly infusion (tea or fermented brew) made from water and organic material (e.g. grass clippings, hay, dead

leaves, yeast, pelleted rabbit chow, horse manure, etc.). When trapping *Culex* species it is best to use a bait infusion made from a recommended formula (see Attachment 3.C for a formula used to make a highly effective gravid trap bait for *Culex* species). Gravid *Culex* mosquitoes are attracted to the smelly water infusion as a place to lay their eggs. There is a higher probability of collecting virus-infected mosquitoes in a gravid trap than in a light trap because gravid traps attract female mosquitoes that have already taken at least one blood meal and are ready to lay eggs. The species collected may vary by where the trap is set and/or what formula is used to make the infusion bait. Traps are best set under bushes, under porches, in tall grass, or out of the wind in areas close to where target vector species may be seeking a place to lay eggs. When trapping any mosquito species, gravid traps are best set sometime between 2:00 and 4:00 PM and collected the next day around 8:00 or 9:00 AM. Gravid traps collect live mosquitoes, and fresh specimens are preferred for arboviral testing; virus isolation by tissue culture works best in mosquitoes that have been dead for less than a day.

b. CDC light trap (Attachment 3.D) – CDC light traps are one of the standard tools for arboviral surveillance. Like the gravid trap, this trap is very portable because it is lightweight and can be powered by a 6-volt lantern battery. The CDC light trap uses a small light source to attract and capture mosquitoes that are seeking a host for a blood meal. Unlike the gravid trap, a CDC type light trap attracts a relatively wide variety of species and because of this, is the best trap to use for identifying the species composition of a locality. The CDC light trap is highly effective for trapping and monitoring various species of floodwater and marsh mosquitoes, but may only be marginally or poorly attractive to other species including *Culex pipiens* which is more attracted to gravid traps. The CDC light trap is the best tool for monitoring *Culiseta melanura*, the primary vector for EEE, and for monitoring many of the important bridge vectors of WNV and EEE. Baiting the trap with CO_2 increases both the number of mosquitoes and range of species collected, as compared to traps using light as the sole attractant. Use of CO_2 to bait the trap requires a supply of dry ice, or canisters of compressed CO_2; a trap baited with CO_2 may require 2-3 pounds of dry ice or compressed gas per night. Mosquitoes are trapped live, and this feature helps maintain the freshness of mosquito specimens that are being tested for arboviruses. CDC light traps use only a small light source that attracts relatively few non-mosquito, insect species such as beetles and moths. It is best not to trap non-mosquito species because they make sorting and identification a lot of work, and/or damage the trapped mosquitoes.

The CDC light trap collects mosquitoes that are mostly undamaged and this makes them easier to identify.

c. New Jersey light trap (See Attachment 3.E) - This trap has historically been a major component of mosquito abatement programs, but is not very useful for arboviral surveillance. These traps use a 25-watt light bulb as an attractant, and a fan draws the insects into a collection jar, which is usually equipped with a vapona strip as a killing agent. One disadvantage of New Jersey light traps is that they are large and heavy, and require standard electrical current. That limits trap placement to locations where there is availability of electricity. Another disadvantage is that many large, non-mosquito insects are attracted to the light bulb used in the New Jersey trap, and these larger insects often damage the collected mosquito specimens badly enough that identification is impossible. Unidentifiable, dead specimens are not useful for arboviral testing. Two important target species for WNV surveillance (C*ulex pipiens* and *Aedes vexans*) are attracted to New Jersey traps, and in areas where these species are the predominant mosquito, a New Jersey light trap can be used to monitor their relative population density over time. New Jersey traps are best used in areas where only a few predominant species occur (e.g., near a salt marsh). In such locations the collected species do not need to be identified and the trap catches only need to be counted to provide relative mosquito numbers, from week to week as a means of directing adult mosquito control activities.

d. Mechanical Aspirators – Powered aspirators are useful tools for collecting adult mosquitoes. Some species of mosquitoes (e.g., certain species in the *Anopheles* and *Culex* genus) do not readily come to traps and aspirating them from their resting areas is the only way to collect them in significant numbers. Aspiration is the best way to collect *Anopheles* mosquito species involved in malaria transmission. All mosquito species rest after taking a blood meal and the only way to capture certain mosquito species while they are blood-fed or gravid is to seek out their resting shelters and aspirate them. Blood-fed or gravid mosquitoes are more likely to be infected with an arbovirus such as WNV or EEE. Mosquito resting places include: foliage of certain plants; building walls, ceilings and eaves; the undersides of bridges; the insides of hollow trees and logs; rodent burrows; and the insides of culverts or sewer pipes. Mosquitoes can also be collected with aspirators when they enter vehicles, or swarm around personnel during trap setting activities. Power aspirators range in size from small hand-held, battery powered units to larger battery or gasoline powered backpack units.

e. Citizen Complaints - If the public is informed about whom to call, citizen complaints about adult mosquito activity, or about potential breeding habitats are useful for mosquito surveillance. Maintaining records of citizen complaints, can contribute toward identification and mapping mosquito problem areas. Citizen complaints can be useful when establishing a new surveillance program in an area where the mosquito breeding habitats and/or areas affected by adult mosquito activity have not yet been identified. Citizen complaints can be mapped as points on a map, and clusters of points will indicate a persistent problem area. Complaints can also be investigated through visitation and direct observation, trapping and/or aspiration of adult mosquitoes and larval dipping in identified habitats.

A sizable portion of citizen complaints are unfounded or misidentify the source of the mosquitoes, so it may take a person with some knowledge of mosquito biology to question the complainant and get the complete and pertinent facts. Obtaining detailed information from the caller (e.g., what time the mosquitoes were active, whether they were biting or not, what their biting behavior was, how large the mosquitoes were, what the mosquitoes looked like, whether there are any suspected breeding grounds near by, etc.) will help screen complaints and avoid unnecessary visitations/investigations. For example, adult mosquitoes are relatively small and are generally difficult to observe, so people do not normally notice them unless they are biting or trying to bite. There are thousands of small flying insect species that might be mistaken for mosquitoes, so if the insects noticed by the complainant were not trying to bite or biting, it is probably unlikely that they were mosquitoes. Questioning the complainant about the time of day mosquitoes were biting is a useful screening tool. For example, Asian tiger mosquitoes are one of relatively few mosquito species that bite during the daytime (daylight hours) and because most complaints in Virginia are related to Asian tiger mosquito activity, determining that the mosquitoes are biting during daylight hours will indicate that the problem mosquitoes are most probably Asian tiger mosquitoes. Also, Asian tiger mosquitoes only breed in containers (**not in puddles or ground pools**) and because they generally do not move far, they probably have originated from a container on the complainants property, or from one that is on a neighbor's property. Therefore, if the complainant is indicating that the mosquitoes are biting during the daylight hours and that they originate from a nearby pond or ditch, the person taking the

complaint will know that the identified habitats are an unlikely source.

3. **Mapping and Analysis of Mosquito Surveillance Data** - Surveillance activities should include locating mosquito breeding habitats and defining the geographic range (area) affected by adult mosquitoes from an identified habitat. Habitats and areas of adult activity can be marked on paper maps and used for reference when planning control activities. The use of Global Positioning System (GPS) devices is recommended for accurate mapping, and is indispensable for mapping with computer based Geographic Information Systems (GIS) software. Use of GIS requires good surveillance data management. It also requires adequate computer hardware and software. GIS mapping allows the incorporation of many map layers that include such information as: road layout, jurisdictional boundaries, human population density, aquatic and/or wetland habitat types, topography, aerial photography indicating vegetation zones, etc. These many map features can aid in the analysis of mosquito data, or in the planning of control programs. Some mosquito control programs use GPS devices linked to GIS maps and to spraying equipment to plan, control, and monitor their insecticide coverage during control operations. Many local governments have planning departments with GIS capabilities, and these departments may be useful as a GIS mapping resource.

4. **Virus Testing of Adult Mosquitoes** – It is not appropriate to submit all mosquito species for arboviral testing. Surveillance programs should concentrate on trapping and submitting approved vector species for testing (see approved list Attachment 3.F). Collected mosquitoes should be pooled for testing. Pools of most approved mosquito species consist of 25 to 50 individual mosquitoes of the same species from the same location and collection date. Certain important vector species may be submitted in pools of as few as 10 mosquitoes. The labs will not test pools containing fewer than 10 mosquitoes unless the submitter has obtained prior approval to submit them. Approval can be obtained by contacting Dr. David Gaines, (Public Health Entomologist, VDH-Office of Epidemiology, Tel. (804) 864-8141; david.gaines@vdh.virginia.gov), and/or by contacting personnel in charge of testing at the laboratories (see Attachment 3.G).

   Pooled mosquitoes should be accurately identified and grouped by species, site, and week of collection. Depending on the county, city or geographic location of your surveillance program pooled mosquitoes should either be sent to the Norfolk Department of Public Health Laboratory (NDPHL) or to the State Division of Consolidated Laboratory Services (DCLS) laboratory in Richmond for testing (see Attachment 3.G). It is best if pool are frozen until shipment or delivery to the laboratory, and shipped in insulated containers packed with dry ice. Target species for laboratory submission vary by disease/pathogen of concern. For WNV, 19 species

are currently tested, and seven species are given priority for testing. For EEE, 14 species are tested and four are given priority. For LAC, four species are tested and three are given priority. Within the *Culex* genus, *Cx. pipiens*, *Cx. salinarius*, *Cx. restuans*, and *Cx. erraticus* are tested. The mosquito *Culiseta melanura* is also tested. The *Aedes* species tested include *Aedes albopictus* (the Asian tiger mosquito) and *Ae. vexans*. *Ochlerotatus* species that breed in containers (i.e., *Oc. atropalpus*, *Oc. triseriatus,* and *Oc. japonicus*) are considered important to test. Several other salt marsh and floodwater *Ochlerotatus* species are also recommended for testing (see Attachment 3.G). The *Culex* species and the *Culiseta melanura* mosquito might all act as important primary vectors (bird feeding species that amplify WNV in the bird population). All of the above species except *Cx. restuans* and *Cs. melanura* may also act as bridge vectors (species that can transmit WNV from birds to humans or other mammals). Except for *Cs. melanura*, and *Cx. erraticus*, all of the above listed species have been tested and proven to have WNV vector competence in laboratory trials. Field collected pools of each of the above listed species have tested positive for WNV in the United States. Early season pool submissions should concentrate on primary vector species involved in amplification of the virus in the bird population. Once WNV has been detected in the local bird or primary vector population, pooling and testing efforts should also concentrate on bridge vector species of mosquitoes.

5. **Surveillance and Testing Data** – The use of a **standardized** database by Virginia's mosquito surveillance programs facilitates compilation of data for the entire state, and comparison of mosquito data from one region to another. When collected mosquitoes are pooled for testing, the use of a standardized database to report those pools to the laboratory facilitates entry and tracking of that data by laboratory personnel as well as the accurate analysis and reporting of test results to the collecting agency, VDH and the CDC.

   A database for the management of adult mosquito surveillance records and submitted mosquito pool records will be available from the VDH in May of 2004. The database written for MSDE (SQL Server software) will be provided free of charge, to all surveillance programs statewide, on a CD-ROM. The operating software (MSDE) the database software and database operating instructions will be included on the CD-ROM. The MSDE software used requires that computers have a Windows 2000 or newer operating system, and will not function properly on older versions of the Windows operating system. Newly organized surveillance programs are encouraged to obtain and use this database for their own mosquito data management. Programs needing a copy of the database should contact Dr. David Gaines, (Public health Entomologist, VDH-Office of Epidemiology, Tel. [804] 864-8141). Programs using the

database should e-mail copies of their weekly mosquito pool data to their mosquito-testing laboratory on, or before the date that pools are sent to the laboratory. Sending an electronic copy of each week's pool data to the laboratory will facilitate laboratory management of test data. Surveillance programs that do not have computer capabilities for using this database may use the Weekly Adult Mosquito Trap Collection, and Pool Submission Forms (found in Attachments 3.B and 3.G). Programs conducting larval surveillance should compile larval surveillance data using the larval surveillance form (Attachment 3.A).

C. WNV Phased Response Plan

The phased response plan provides recommended levels of surveillance activity, and recommended surveillance activities for each level of WNV activity detected in a geographic region. Each jurisdiction should define its own geographic surveillance region based on human population density, topography, road layout or other locally important geographic features. (See the Avian Surveillance, Section C. 6. of the WNV Plan)

1. **Level Ia** – Winter weather, low likelihood of WNV epizootic activity, and little or no adult mosquito vector activity present.

a. Larval surveillance – Use maps, windshield surveys and walking surveys or aerial surveillance to identify and map the locations of wet or flood prone areas, tire piles and other potential mosquito breeding habitats. The absence of foliage on trees or shrubs during the winter season facilitates the observation and identification of potential mosquito breeding habitats from the road or from the sky.

b. Adult mosquito surveillance – Analyze and map previous year's citizen complaint calls and surveillance records of mosquito activity to help determine future target locations for surveillance and control activities.

2. **Level Ib** – Early mosquito breeding season (April – May), adult mosquito activity present, no current evidence of WNV epizootic activity.

a. Larval surveillance – Use investigative surveillance (larval dipping) in suspected habitats to identify mosquito breeding sites. Identify neighborhoods where container breeding mosquitoes are a problem.

b. Adult mosquito surveillance – Use investigative trapping and collections in suspected and identified problem areas to identify the species present and to monitor for vector species. Target surveillance to detect primary vector species (*Culex* species) by

extensive use of gravid traps and CDC-light traps. Commence pooling and testing of mosquitoes, focusing on primary vector species only. There is little need to be testing bridge vector species at this time.

3. **Level II** – Early Summer (June – July), mosquito larvae and adults present, initial evidence of WNV epizootic activity in region (limited to birds or mosquitoes).

   a. Focus mosquito surveillance efforts in areas close to where WNV infected birds have been found. Clusters of WNV infected birds are an indication of considerable WNV activity in primary vector species. Primary vectors include *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*, (some other species such as *Culiseta melanura* or even *Cx. erraticus* might also merit investigation even though their importance as WNV vectors has not yet been established). Surveillance efforts should focus on detecting the habitats of the primary vectors for control purposes. Collected adults of primary vector mosquito species should be tested to measure their WNV infection rates.

   b. Larval surveillance: Seek out habitats and conduct investigative larval dipping to identify primary vector habitats in the vicinity of WNV positive bird finds. If possible, continue to conduct regularly scheduled (weekly) larval mosquito surveillance in other identified breeding habitats.

   c. Adult Surveillance: Use both gravid traps and CDC-light traps to detect and test primary vector species in areas close to WNV positive bird finds. Gravid traps are the best means of capturing *Cx. pipiens* or *Cx. restuans*, and CDC light traps are the best means of collecting the other potential primary vector species (*Cx. salinarius*, *Cx. erraticus* and *Cs. melanura*). If possible, continue to conduct regularly scheduled (weekly) adult mosquito surveillance at other identified problem areas using light traps and gravid traps. Commence to focus attention on suspected important bridge vector species such as *Aedes albopictus*, *Ae. vexans*, *Ochlerotatus triseriatus* and *Oc. japonicus*. Submit pooled vector mosquito species to laboratory for testing.

4. **Level III** – Mid-summer to Fall, moderate WNV epizootic activity in mosquitoes and birds with initial evidence of WNV in a horse or human.

   Increase larval and adult mosquito surveillance in identified areas of WNV epizootic activity. Continue use of both gravid and CDC type traps and increase pooling and testing of both primary vector and important bridge

vector species. Investigate and test mosquito populations in the vicinity of all equine and human cases.

5. **Level IV** – Mid-summer to Fall, WNV epizootic activity suggesting high risk of human infection (i.e., high dead and/or positive bird densities, high mosquito infection rates). confirmed human or horse case, abundant adult bridge vectors.

   Maintain heightened levels of larval and adult mosquito surveillance and adult mosquito testing. Focus more adult surveillance efforts on populations of potential bridge vector species in areas where WNV activity has been found in primary vector species, horses and humans.

6. **Level V** – Mid-summer to Fall, multiple human cases of WNV and conditions favoring further transmission to humans.

   Maintain heightened levels of larval and adult mosquito surveillance and adult mosquito testing. Where possible, investigate mosquito populations (species and infection rates) in the vicinity of where identified human cases were thought to have been exposed to mosquitoes.